

**DEPTH AND PERCENTAGE OF PENETRATION OF BIOCERAMIC SEALER
INTO THE DENTINAL TUBULES USING ULTRASONIC, ROTARY, AND
CONVENTIONAL SEALER PLACEMENT TECHNIQUE- A CONFOCAL
LASER SCANNING MICROSCOPIC STUDY**

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In partial fulfilment for the degree of

MASTER OF DENTAL SURGERY



BRANCH – IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

APRIL 2013- 2016

ENDORSEMENT BY THE H.O.D. PRINCIPAL / THE HEAD OF THE INSTITUTION

This is to certify that **Dr.S.KUMAR**, Post Graduate student (2013–2016) in the Department of Conservative Dentistry and Endodontics, K.S.R. Institute of Dental Science and Research, has done this dissertation titled **“DEPTH AND PERCENTAGE OF PENETRATION OF BIOCERAMIC SEALER INTO DENTINAL TUBULES USING ULTRASONIC, ROTARY, AND CONVENTIONAL SEALER PLACEMENT TECHNIQUE- A CONFOCAL LASER SCANNING MICROSCOPIC STUDY”** under our guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University**, Chennai – 600 032 for **M.D.S.,** (Branch – IV) **CONSERVATIVE DENTISTRY AND ENDODONTICS** degree examination.

Seal & Signature of H.O.D.

Dr.K. SIVAKUMAR

PROFESSOR

Seal & Signature of Principal

Dr. G.S. KUMAR.,M.D.S

PRINCIPAL

CERTIFICATE BY THE GUIDE

This is to certify that dissertation titled “**DEPTH AND PERCENTAGE OF PENETRATION OF BIOCERAMIC SEALER INTO DENTINAL TUBULES USING ULTRASONIC, ROTARY, AND CONVENTIONAL SEALER PLACEMENT TECHNIQUE - A CONFOCAL LASER SCANNING MICROSCOPIC STUDY**” is a bonfide research work done by **Dr.S.KUMAR** in partial fulfillment of the requirements for the degree of **MASTER OF DENTAL SURGERY** in the specialty of **CONSERVATIVE DENTISTRY AND ENDODONTICS**

Date:

Signature of the Guide

Place:

DR.SEBEENA MATHEW., M.D.S

PROFESSOR

K.S.R. INSTITUTE OF DENTAL SCIENCE AND RESEARCH

TIRUCHENGODE

DECLARATION BY THE CANDIDATE

TITLE OF DISSERTATION	Depth and percentage of penetration of Bioceramic sealer into dentinal tubules using ultrasonic, rotary and conventional sealer placement technique - a confocal laser scanning microscopic study
PLACE OF STUDY	K.S.R. Institute of Dental Science and Research
DURATION OF THE COURSE	3 Years
NAME OF THE GUIDE	Dr. Sebeena Mathew
HEAD OF THE DEPARTMENT	Dr. Sivakumar

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Signature of the candidate

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INTRODUCTION

INTRODUCTION:

A three dimensionally obturated root canal system prevents percolation and microleakage of periapical exudate into the root canal space. It prevents reinfection and creates a favourable environment for healing to take place ^[1]. Though endodontic success requires elimination of micro-organisms through mechanical instrumentation, antibacterial irrigants, and use of antimicrobial dressings and the adequate filling of the empty space, complete sterility is achieved when these procedures are coupled with root canal sealers that have an antimicrobial effect ^[2]. Thorough chemo mechanical preparation has to be coupled with a well obturated root canal space, to prevent apical or coronal leakage and to entomb residual debris and recalcitrant bacteria ^[3]. The obturating materials and the techniques used to place them, must achieve a high level of adaptability to the cleaned root canal space and dentinal walls, including penetration into the dentinal tubules if possible^[4].

The most commonly used core filling material is gutta-percha. Although gutta-percha can be reasonably adapted to the root canal walls, because of the canal irregularities and the size of the dentinal tubules, a root canal sealer is essential to not only assist in filling irregular spaces, but also to enhance the seal during compaction and to penetrate into small, normally inaccessible areas, i.e., the dentinal tubules^[5]. The penetration of sealer cements into dentinal tubules is considered to be a desirable outcome for a number of reasons: It increases the interface between the guttapercha and dentinal walls, thus improving the sealing ability and retention of the material. The retention of the obturating materials can be improved by mechanical interlocking. The penetration of sealer cements into dentinal tubules may also entomb any residual bacteria within the tubules and the chemical components of sealer cements may exert an antibacterial effect that will be enhanced by closer approximation to the bacteria. Therefore, it is important that the percentage of the sealer/dentin interface

covered by the sealer and the degree of tubule penetration by the sealer be as great as possible in all cases, whether previously infected or not^[6].

Placement of a sealer into the root canal system should be done in a manner which is predictable and completely covers the dentin walls ^[7]. Accepted means of sealer placement include the use of endodontic files or reamers, lentulospirals, gutta-percha cones, paper points, and recently ultrasonic files. The analysis of the dentin/sealer interface allows the determination of which filling technique could obturate the root canals with less gaps, voids and evenly coats the thin layer of the sealer into the canal ^[8]. Several microscopy techniques are currently used to evaluate the sealer/dentin interface, including stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal laser scanning microscopy (CLSM) ^[9]. In comparison to conventional SEM, CLSM has the advantage of providing detailed information about the presence and distribution of sealers or dentinal adhesives inside dentinal tubules, in the local circumference of the root canal walls, at relative low magnification and non-dehydrated samples through the use of fluorescent Rhodamine-marked sealer ^[10]. Therefore, the purpose of this study was to compare the depth of three different placement techniques using CLSM as the evaluative tool.

AIMS & OBJECTIVES

AIM:

To compare the depth and percentage of sealer penetration into dentinal tubules with Ultrasonic, rotary and gutta percha sealer placement techniques using confocal laser scanning microscopy.

OBJECTIVES:

The main objective is to

- Evaluate the Depth of penetration of bio-ceramic sealer into dentinal tubules by Ultrasonic, lentulospiral, Gutta percha sealer placement techniques.
- Evaluate the Percentage of Penetration to check for uniform distribution of bioceramic sealer along the dentinal walls by ultrasonic, lentulo and guttapercha sealer placement techniques.

REVIEW OF LITERATURE

REVIEW OF LITERATURE:

Three-dimensional obturation of the root canal system is the final objective of non-surgical root canal therapy. The most commonly used core-filling material is gutta-percha, but it has the disadvantage of being non-adherent to canal walls. In addition presence of canal irregularities and the size of the dentinal tubules a root canal sealer is essential to enhance the seal during compaction and to penetrate into small, normally inaccessible areas, i.e., the dentinal tubules. The penetration of sealer cements into dentinal tubules is considered to be a desirable outcome for a number of reasons: It will increase the interface between material and dentin thus improving the sealing ability and retention of the material may be improved by mechanical locking. Sealer cements within dentinal tubules may also entomb any residual bacteria within the tubules and the chemical components of sealer cements may exert an antibacterial effect that will be enhanced by closer approximation to the bacteria. Therefore, it is important that the percentage of the sealer/dentin interface that is covered by the sealer and the degree of tubule penetration by the sealer be as great as possible in all cases, whether previously infected or not.

Guldener (1985) suggested choosing a working length which corresponds to the tooth length less 0.5mm for cases with a necrotic pulp. In cases of vital pulp extirpation, he recommended an additional reduction of 0.5 mm, 1mm short of the tooth length^[11].

Frank et al. (1988) suggested an apical stop located between 0.5 mm and 1 mm from the apex. The stop represents the apical limit of the instrumentation and obturation^[12].

Weine. (1996) advocated enlarging the apical part of the root canal to three sizes larger than where the first file bound. But other authors have concluded that it is questionable whether, filing three sizes larger than the first file that binds will adequately remove dentin circumferentially in the canal^[13, 14].

Ingle (2008), **Torabinejad** (2009) stated that pertinent aim of root canal treatment is to do away with the microbial entity and any future predilection of reinfection. In order to achieve this, proper seal is required to denigrate any chance of proliferation of bacteria and future occurrence of any pathology. Sealer along with solid obturating material acts synergistically to create a hermetic seal. ^[15, 16]

Schafer (2003) the quality of the seal obtained with guttapercha (GP) and conventional zinc oxide eugenol (ZOE) sealers is quite far from being perfect. ^[17]

Texieria (2004) **Hammad** (2007) Gutta Percha and resin based sealer combination still has its own shortcomings, like its inability to reinforce the root. It does not adhere to dentin. Resin based sealers are not able to control microleakage. The solubility of sealer makes prognosis dilemmatic and un-assuring. Although few materials are capable enough to swap GP on multiple parameters, research continues to find alternatives that may seal better and mechanically reinforce compromised roots by forming a monoblock, which has been suggested to reduce bacterial ingress pathways and strengthen the root to some extent. ^[18, 19]

Bouillaguet (2008) New resin cement sealants like AH plus, Gutta flow, Epiphany have been developed to be used instead of ZOE, thereby improving the root canal seal and imparting more strength as compared to the conventional materials. ^[20]

Poggio (2011) Mineral trioxide aggregate (MTA) -based sealers have the predilection toward mineralization along with all the viable properties of orthodox sealers. ^[21]

Camilleri (2005) **Gomes-Filho JE** (2009) Sealers based on MTA have been reported to be biocompatible, stimulate mineralization, ^[22] and encourage apatite-like crystalline deposits along the apical- and middle-thirds of canal walls. ^[23]

Huffman (2009) MTA based sealer exhibited higher push-out bond strengths after storage in simulated body fluid ^[24] and had similar sealing properties to epoxy resin-based sealer, when evaluated using the fluid filtration system.^[22]

Sagsen (2011) assessed the push out bond strengths of two new calcium silicate-based endodontic sealers, MTA Fillapex and iRoot SP and compared them with AH plus in the root canals of extracted teeth and found that in the coronal specimens, there was no significant difference between the sealers. In the middle and apical segments, there was no significant difference between iRoot SP and AH Plus groups. However, the iRoot SP had significantly higher bond strength values than the MTA Fillapex. So, they concluded that MTA Fillapex had the lowest push-out bond values to root dentine compared with other sealers. ^[25]

Yang Q (2008) the major inorganic components of Bioceramic sealer include tricalcium silicate, dicalcium silicate, calcium phosphates, colloidal silica, and calcium hydroxide. It uses zirconium oxide as the radiopacifier and contains water-free thickening vehicles to enable the sealer to be delivered in the form of a premixed paste. ^[26]

Yang Q (2002) Hydroxyapatite is co-precipitated within the calcium silicate hydrate phase to produce a composite-like structure, reinforcing the set cement. ^[27]

Xu H (2007) Premixed calcium phosphate silicate-based sealer eliminates the potential of heterogeneous consistency during on-site mixing. Because the sealer is premixed with non-aqueous but water-miscible carriers, the water-free paste will not set during storage in the syringe and only hardens on exposure to an aqueous environment. ^[28]

Zhang (2009) the pH of EndoSequence BC Sealer during the setting process is higher than 12, which increases its bactericidal properties. ^[29]

Candeiro (2012) the amount of Ca^{2+} released from EndoSequence BC Sealer was far higher (2.585 mg/l) than that released from AH Plus (0.797 mg/l), mainly after 7 days.^[30]

Zoufan (2011) conducted a study which evaluated the cytotoxicity of GuttaFlow and EndoSequence BC sealers and compared them with AH Plus and Tubli-Seal sealers. The GuttaFlow and EndoSequence BC sealers had lower cytotoxicity than the AH Plus and Tubli-Seal sealers.^[31]

Deyan Kossev and Valeri Stefanov (2009) found that when bioceramic-based sealers BioAggregate or iRoot SP are extruded, the pain is relatively small or totally absent. Such lack of pain may be explained based on the characteristics of these new materials. During hardening, they “produce” hydroxylapatite and after the end of hardening process they exhibit the same features as non-resorbable hydroxylapatite-based bioceramics used for bone replacement in oral surgery. Due to the hydroxylapatite formed, they are also osseo-conductive. During setting, hard ceramic-based sealers expand. Expansion of BioAggregate and iRoot SP and iRoot BP is significant (0.20%). These new bioceramic sealers also form chemical bond with the canal’s dentin walls. That is why no space is left between the sealer and dentin walls.^[32]

Hoan (1988) Placement of a sealer into the root canal system should be done in a manner which is predictable and completely covers the dentin walls.^[33]

Wiemann (1988) Several techniques of sealer placement have been described in the literature, such as the use of a file, lentulo spiral, absorbent paper point, gutta-percha cone, and an ultrasonic file. Each technique may produce different distribution of the sealer onto the canal walls, which may affect the sealing.^[33, 34]

Gutmann (1993) To achieve the goal of thorough canal obturation, not only must the tissue debris and contaminants be removed, but also the filling materials and techniques used to place them must achieve a high level of adaptability to the cleaned root canal space and dentin walls, including penetration into the dentinal tubules if possible.^[35]

Alphino (2006) confocal laser scanning microscopy (CLSM) was used to obtain a series of optical XY images that were recorded through the thickness of the dentin. Compared with scanning electron microscopic and histologic methods, confocal microscopy has the advantage of providing detailed information about the presence and distribution of sealers or dental adhesives inside dentinal tubules in the total circumference of the root canal walls at magnification as low X50- X100 through the use of fluorescent rhodamine– marked sealers.

[10]

MATERIALS AND METHODS

ARMAMENTARIUM USED:

1. Extracted Maxillary anterior teeth
2. 0.3% thymol solution
3. Diamond disc and mandrel
4. Airotor Handpiece (NSK)
5. Micromotor handpiece (NSK)
6. K files (VDW, Germany)
7. Saline
8. 5.25% sodium hypochlorite.
9. Disposable syringe (Dispovan)
10. X-smart (Dentsply mallifer)
11. Protaper rotary files (Dentsply mallifer)
12. Protaper Gutta percha F3 size (Dentsply mallifer)
13. Endosequence BC sealer (Brasseler USA)
14. U-Files
15. Scaler Unit (Satlec)
16. Lentulospiral (Mani.Inc)
17. Rhodamine B Dye.
20. Cavit-on (GC corporation)

21. Hand Plugger.

SOURCE OF DATA:

Extracted Maxillary anterior has been collected from Department of Oral-maxillofacial surgery in KSR Institute of dental science and research, study has conducted in Department of Conservative Dentistry And Endodontics in KSR Institute of dental science and research. Confocal laser scanning microscopic Imaging has been done In Central Manufacturing technology Institute, Tumkur Road, Bengaluru and Vclin Bio Research centre, Sri Ramachandra University, Chennai.

INCLUSION CRITERIA:

- Uni-radicular teeth.
- Teeth with single root canal.
- Teeth with circular shape canal.

EXCLUSION CRITERIA:

- Multiradicular teeth.
- Teeth with multiple canals.
- Teeth with oval or ribbon shaped canals.
- Teeth with any anomalies.
- Teeth with root resorption.

MATERIALS AND METHODS:

Sixty maxillary single-rooted teeth stored in thymol solution were used in this study. The coronal portions were cut with a 0.3-mm diamond disc and the root canal length was standardized at 14 mm. Radiographs were exposed from facial and proximal views to ensure the presence of a single canal.

Root canal preparation

A size 10 K-file was introduced into each canal until it could be seen through the apical foramen and the length measured. Working length was established by subtracting 0.5 mm from that length. Then, the roots were instrumented by using the Protaper technique to a size of F3. The hand piece was used with an electric engine (X-smart, Dentsply-Maillefer, Ballaigues, Switzerland) at 250 rpm. Irrigation procedures were accomplished by using 2mL of 5.25% sodium hypochlorite for each file used. To remove the smear layer, all canals were irrigated with 3 mL 17% EDTA over 2 minutes followed by 2 mL 1.0% sodium hypochlorite over 1 minute. Finally, the root canals were flushed with distilled water and canals were dried with sterile paper points, Master cone selected using Protaper F3 size Guttapercha. The instrumented teeth were divided randomly into 3 experimental groups of 20 teeth each according to sealer placement techniques:

Group I: Endosequence BC sealer was placed inside the root canal with rhodamine B dye at concentration of 0.1% using disposable tips. The root canal sealer is activated using ultrasonic file for 10 seconds. Obturation was done using Single cone method. Excess Guttapercha was condensed using a heated plugger.

Group II: Lentulo spiral was selected that would not bind in the prepared canal and that would reach the prepared working length. After the hand piece was set to enable the lentulo

spiral to spin the material apically, the 0.05 ml of Endosequence BC sealer was placed into the canal and was gently rotated to working length and worked gently up and down within the canal for at least 5 seconds. Obturation was done using Single cone method. Excess Guttapercha was condensed using a heated plugger.

Group III: Endosequence BC sealer was placed inside the root canal with rhodamine B dye at concentration of 0.1% using disposable tips. Master cone coated with sealer and then placed inside the canal. Obturation done using Single cone method. Excess Guttapercha was condensed using a heated plugger.

Extruded sealer from apical foramen, if present, was wiped off with moist cotton. Coronal end of root canal was sealed with Cavit-on temporary paste. All the roots were stored in container at 100% humidity and 37°C for 7 days to allow the sealer to set.

Sectioning and image analysis

The roots were sectioned using a diamond disc at 200 rpm and continuous water cooling to prevent frictional heat. Horizontal sections were done at the 3,6 and 9 mm levels from the apical foramen. Then, the surface were polished using sand papers number 500, 700, and 1200 under running water to eliminate debris product of the cutting procedure. The samples submitted to confocal laser microscopy had 2 mm thickness. The dentin segments were examined on a confocal microscope (Olympus Fluoview FV 1000). The respective absorption and emission wave lengths for the Rhodamine B were 540 nm. Dentin samples were analyzed using the 10× lens for percentage of penetration and 20 × lens for depth of penetration into dentinal tubules.

To calculate the percentage of sealer penetration around the root canal, first each image was imported into the IOB software and the circumference of root canal measured.

Next, areas along the canal walls in which the sealer penetrated into dentinal tubules were outlined and measured using the same method. Subsequently, the percentage of root canal sealer penetration in that section was established. The canal wall served as the starting point and sealer penetration into dentinal tubules was measured to a maximum depth of 1,000 μm . These data points were averaged to obtain a single measure for each section. Statistical significance for the mean of depth penetration of root canal sealers was determined for each level of the root canal using ANOVA followed by Tukey HSD test; the level of significance was set at $P < 0.05$.



Figure 1: Armamentarium used



Figure 2: Armamentarium used

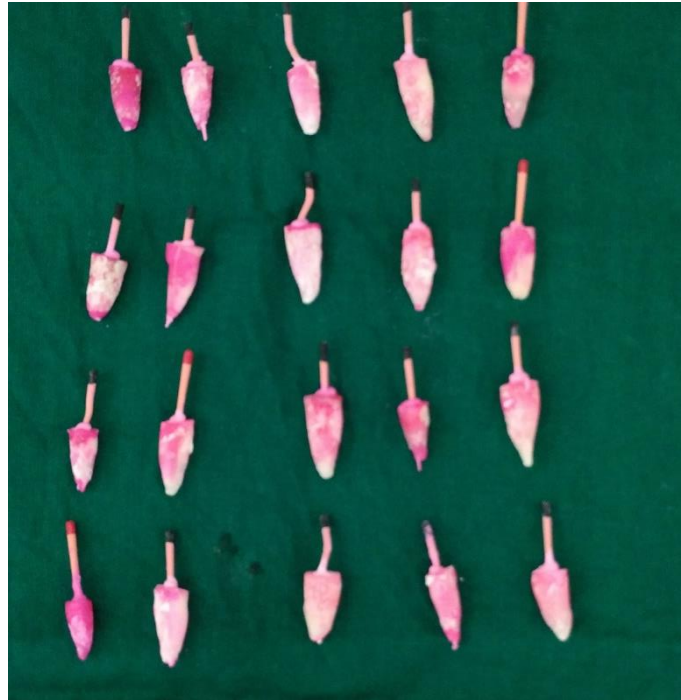


Figure 3: Teeth samples used in the study

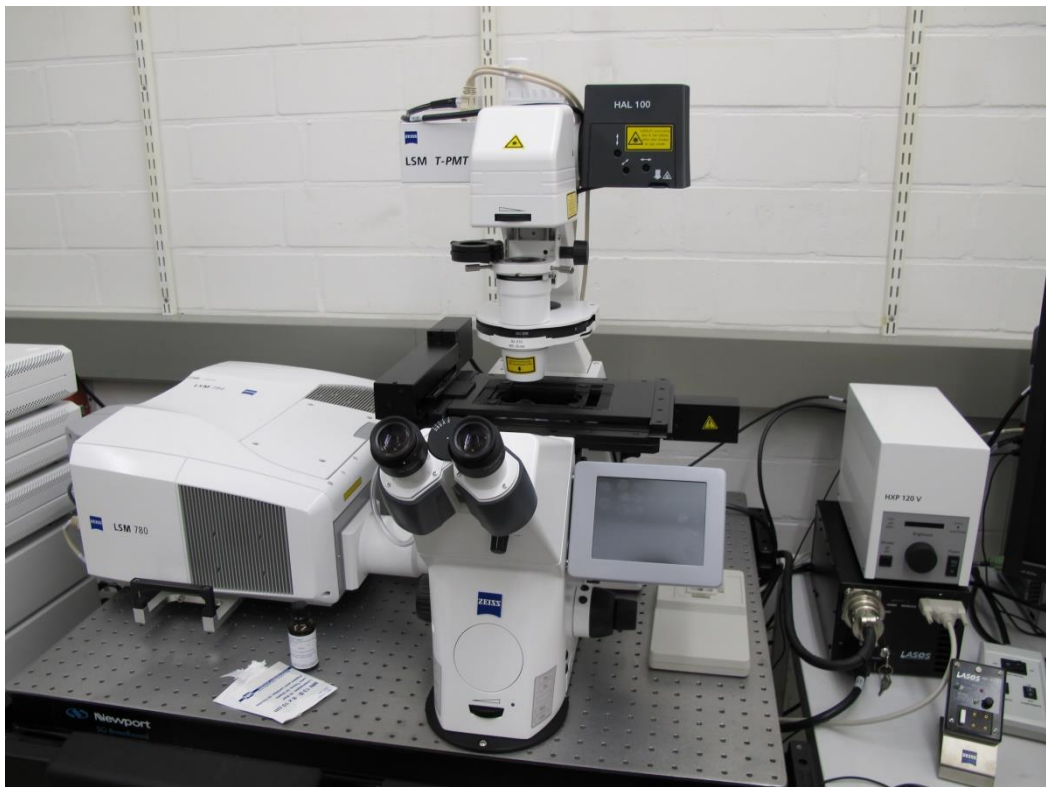


Figure 4: Confocal laser scanning microscope

RESULTS

Table 1: Depth of penetration of Sealers into the dentinal tubules (in μm)

Sample	Ultrasonic	Lentulo	Guttapercha
1c	525.71	512.86	284.53
1m	496.84	489.56	228.95
1a	487.07	472.02	185.02
2c	490.96	512.44	387.99
2m	444.72	487.74	335.87
2a	438.71	422.52	298.54
3c	568.72	528.43	334.85
3m	495.86	499.5	287.45
3a	593.91	480.54	245.64
4c	491.72	551.21	298.78
4m	446.95	516.81	254.13
4a	443.55	493.52	212.41
5c	602.47	452.23	214.58
5m	487.6	437.54	187.45
5a	373.64	398.22	154.2
6c	516.21	441.68	365.54
6m	478.73	412.15	287.52
6a	448.04	402.1	237.08
7c	568.29	568.14	354.09
7m	519.23	538.25	302.24
7a	499.21	511.32	264.78
8c	518.42	487.98	287.54
8m	441.48	479.56	246.87
8a	384.69	456.21	197.63
9c	586.61	661.48	389.54
9m	534.04	614.05	345.78
9a	517.7	598.56	297.54
10c	560.95	594.21	297.54
10m	517.17	523.61	238.54
10a	486.78	495.65	202.41
11c	531.76	487.64	198.78
11m	512	439.7	154.58
11a	492.48	402.57	101.42
12c	557.32	465.21	187.45
12m	532.66	432.87	149.22
12a	463.57	398.71	111.33
13c	565.37	562.31	189.62
13m	463.47	587.91	172.14
13a	457.46	537.46	134.52
14c	565.37	421.37	229.84
14m	485.16	401.64	194.71
14a	457.65	387.56	164.52

15c	591.36	594.86	127.95
15m	573	578.79	108.52
15a	479.02	512.37	95.2
16c	499.69	387.54	147.67
16m	465.34	356.12	127.52
16a	433.69	354	104.78
17c	496.33	473.44	287.54
17m	475.33	420.54	237.91
17a	408.75	392.45	147.2
18c	559.38	476.52	163.54
18m	491.04	439.21	128.52
18a	449.22	403.12	94.13
19c	464.4	531.98	112.23
19m	463.2	502.62	87.42
19a	420.86	487.54	64.08
20c	496.88	462.11	147.54
20m	395.06	437.89	118.87
20a	378.61	411.03	54.12
21c	609.91	557.83	87.52
21m	550.96	523.87	63.21
21a	509.8	499.62	23.12
22c	572.82	465.13	54.12
22m	534.17	418.89	38.74
22a	497.9	402.15	12.14
23c	553.42	438.87	34.25
23m	504.57	405.69	18.45
23a	468.65	387.79	8.05
24c	650.24	396.54	64.52
24m	608.14	354.12	37.97
24a	596.78	322.54	14.78
25c	554.37	467.87	58.25
25m	499.9	442.53	42.68
25a	478.35	421	22.44
26c	578.43	418.95	34.24
26m	542.66	398.22	7.08
26a	498.88	362.13	0
27c	588.64	498.08	0
27m	532.17	462.33	0
27a	434.93	416.2	0
28c	543.12	534.18	6.54
28m	496.87	507.25	8.09
28a	454.64	489.89	0
29c	532.43	466.89	112.54
29m	488.58	423.87	84.13

29a	414.78	402.1	53.12
30c	596.78	462.58	98.52
30m	554.43	438.27	52.13
30a	417.58	398.78	18.2

C- Cervical

M- Middle

A- Apical

Depth of Penetration values are in μm

Table 2: Percentage of Penetration of Root canal sealers (in %)

sample	Ultrasonic	Lentulo	Gutta-percha
1c	94	93	70
1m	87	85	62
1a	83	80	45
2c	96	85	63
2m	92	81	52
2a	87	73	32
3c	98	83	44
3m	96	68	42
3a	93	78	32
4c	92	96	68
4m	88	87	53
4a	84	80	41
5c	88	80	67
5m	79	72	32
5a	69	65	18
6c	94	84	61
6m	85	73	53
6a	81	65	25
7c	98	88	43
7m	93	76	29
7a	87	71	18
8c	97	63	54
8m	88	69	37
8a	81	63	20
9c	95	95	48
9m	95	96	23
9a	92	70	16
10c	91	97	28
10m	86	86	16
10a	79	80	8
11c	82	83	65
11m	72	67	54
11a	84	69	50
12c	83	68	62
12m	72	54	43
12a	82	60	37
13c	98	53	58
13m	84	42	44
13a	85	37	26
14c	84	82	82

14m	79	87	79
14a	76	63	61
15c	91	89	64
15m	81	76	22
15a	77	59	12
16c	72	93	81
16m	69	87	72
16a	67	79	57
17c	89	90	70
17m	71	86	61
17a	76	80	29
18c	98	86	55
18m	93	79	39
18a	81	63	19
19c	88	79	17
19m	73	67	12
19a	70	54	0
20c	82	76	79
20m	79	69	54
20a	73	65	50
21c	99	98	67
21m	96	89	46
21a	81	78	12
22c	90	82	39
22m	80	69	17
22a	67	55	7
23c	91	85	29
23m	76	57	13
23a	69	49	6
24c	95	76	72
24m	91	69	47
24a	87	43	39
25c	89	88	56
25m	82	69	32
25a	76	55	19
26c	96	93	39
26m	83	77	25
26a	79	69	0
27c	91	79	0
27m	88	65	0
27a	73	61	0
28c	89	88	6
28m	81	86	8
28a	77	71	0

29c	95	94	76
29m	85	78	59
29a	77	71	45
30c	95	88	64
30m	84	72	48
30a	79	51	30

C- Cervical

M- Middle

A- Apical

Percentage of penetration- values are in %

Table-1 shows the values obtained in our study for the depth of penetration of root canal sealers into dentinal tubules, **Table-2** shows the values obtained in our study for percentage of penetration of root canal sealers into dentinal tubules.

Figure-5 shows the image of Group I (Ultrasonic) which was obtained in coronal portion for the depth of penetration of root canal sealers into dentinal tubules.

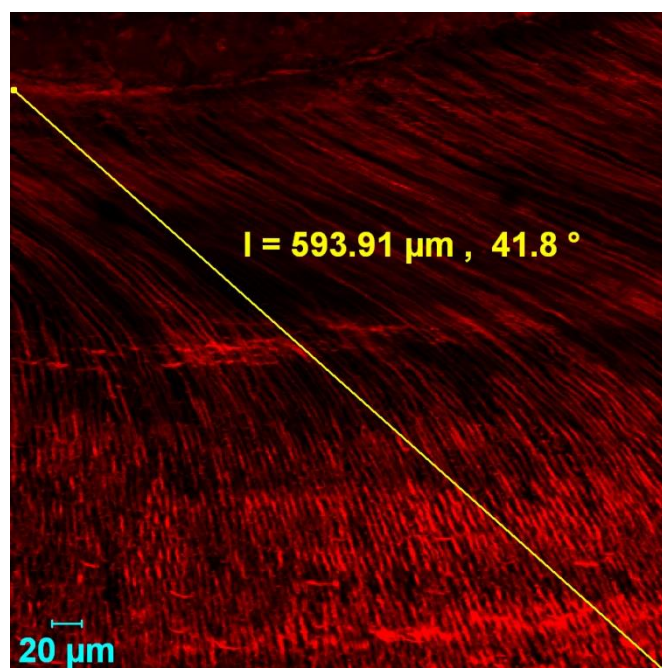


Figure 5-Depth of penetration of root canal sealers into dentinal tubules in the coronal portion of Ultrasonic group.

Figure-6 shows the image of Group I (Ultrasonic) which was obtained in the middle portion for the depth of penetration of root canal sealers into dentinal tubules.

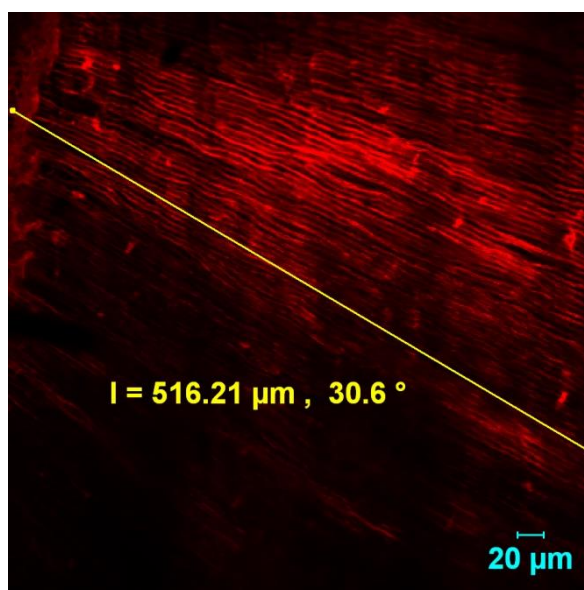


Figure 6- Depth of penetration of root canal sealers into dentinal tubules in the middle portion of Ultrasonic group.

Figure-7 shows the image of Group I (Ultrasonic) which was obtained in the apical portion for the depth of penetration of root canal sealers into dentinal tubules.

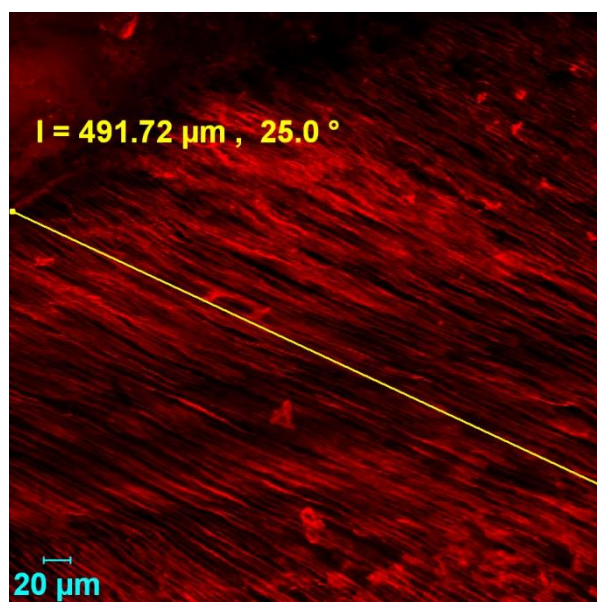


Figure 7- Depth of penetration of root canal sealers into dentinal tubules in the apical portion of Ultrasonic group.

Figure-8 shows the image of Group II (Lentulo) which was obtained in the coronal portion for the depth of penetration of root canal sealers into dentinal tubules.

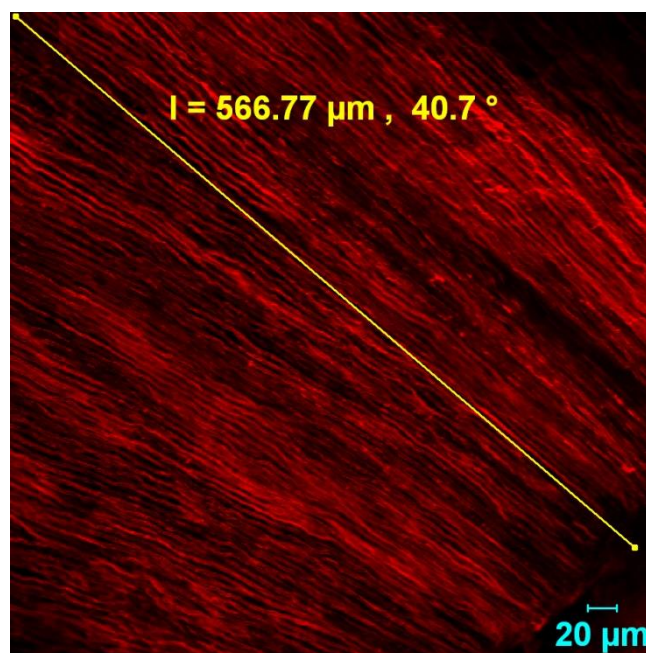


Figure 8- Depth of penetration of root canal sealers into dentinal tubules in the coronal portion of Lentulo group.

Figure-9 shows the image of Group II (Lentulo) which was obtained in the middle portion for depth of penetration of root canal sealers into dentinal tubules.

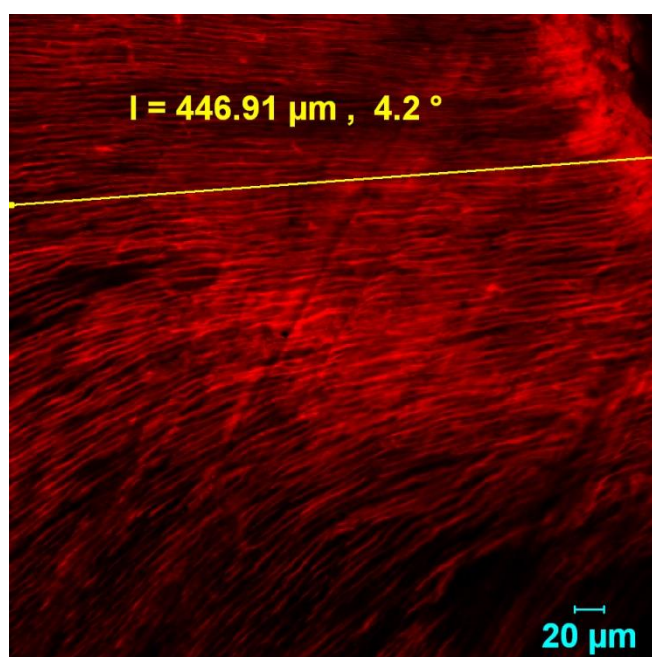


Figure 9- Depth of penetration of root canal sealers into dentinal tubules in the middle portion of Lentulo group.

Figure-10 shows the image of Group II (Lentulo) which was obtained in the apical portion for the depth of penetration of root canal sealers into dentinal tubules.

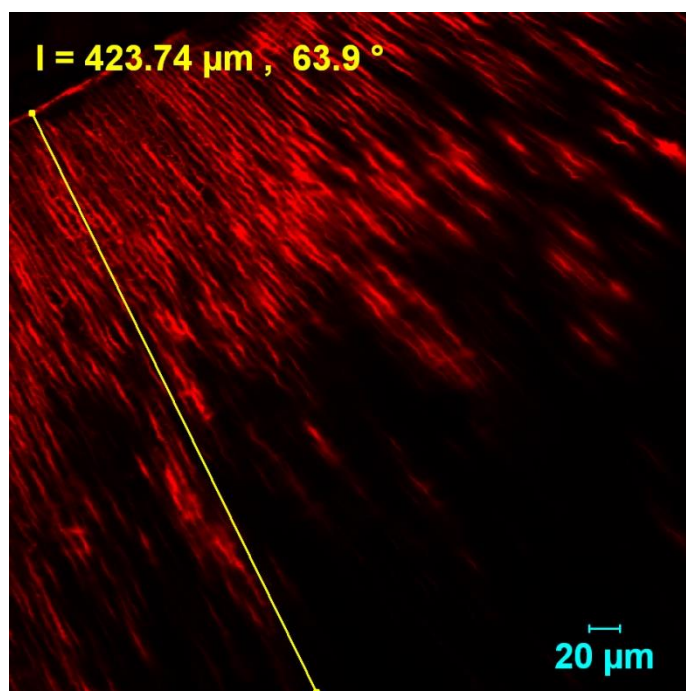


Figure 10- Depth of penetration of root canal sealers into dentinal tubules in the apical portion of Lentulo group.

Figure-11 shows the image of Group III (Gutta percha) which was obtained in the coronal portion for the depth of penetration of root canal sealers into dentinal tubules.

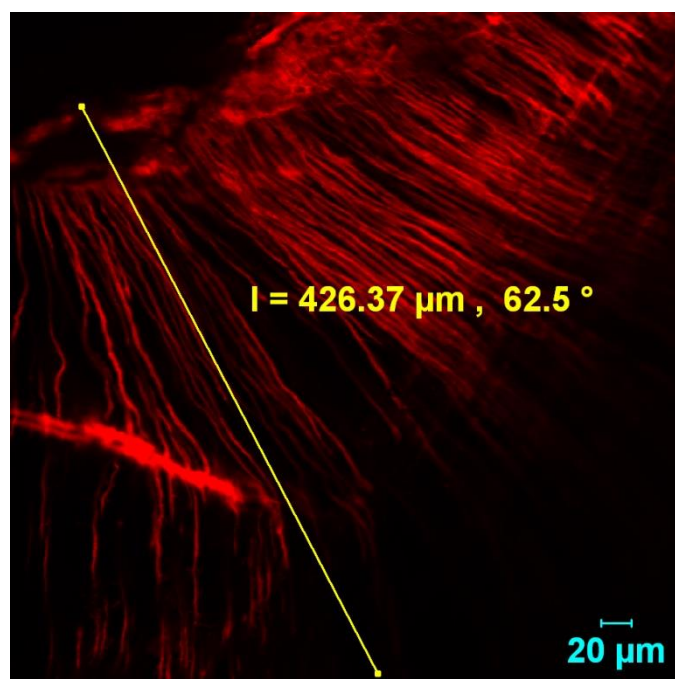


Figure 11- Depth of penetration of root canal sealers into dentinal tubules in the coronal portion of Guttapercha group.

Figure-12 shows the image of Group III (Gutta percha) which was obtained in the middle portion for the depth of penetration of root canal sealers into dentinal tubules.

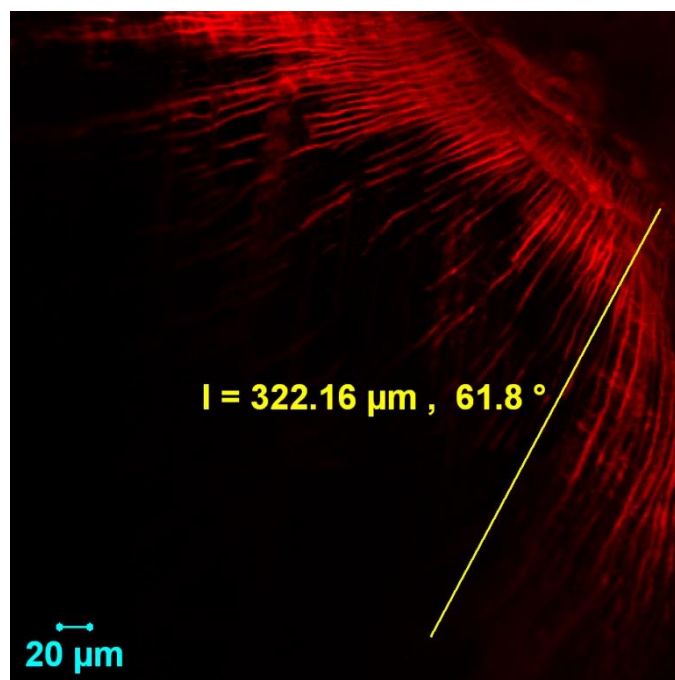


Figure 12- Depth of penetration of root canal sealers into dentinal tubules in the middle portion of Guttapercha group.

Figure-13 shows the image of Group III (Gutta percha) which was obtained in the apical portion for the depth of penetration of the root canal sealers into dentinal tubules.

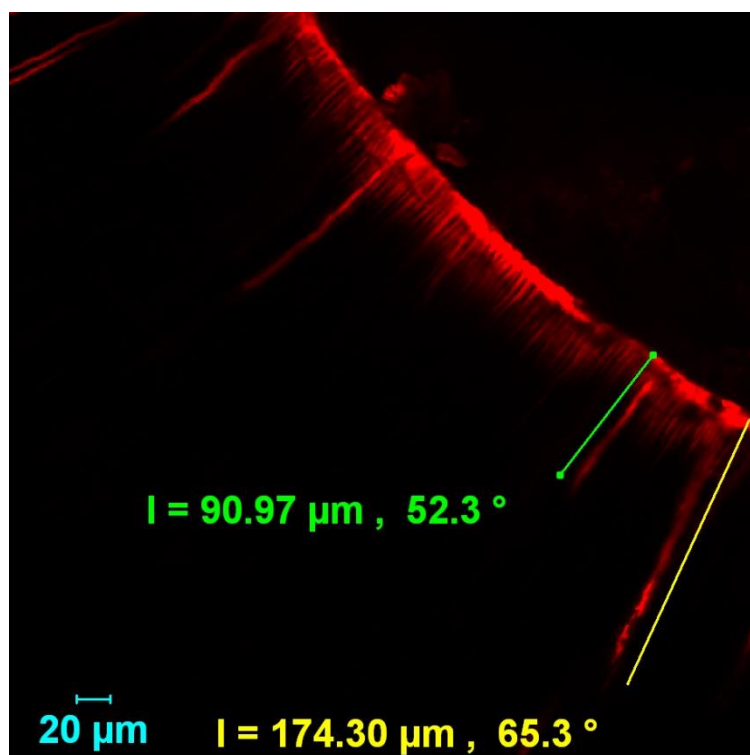


Figure 13- Depth of penetration of root canal sealers into dentinal tubules in the apical portion of Guttapercha group.

Figure-14 shows the image of Group I (Ultrasonic) which was obtained in the coronal portion for the percentage of penetration of root canal sealers into dentinal tubules.

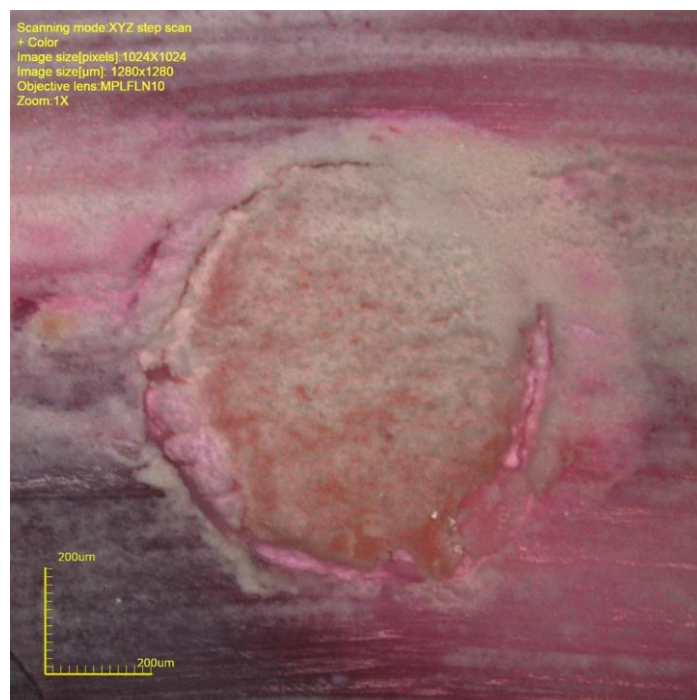


Figure 14- Percentage of penetration of root canal sealers into dentinal tubules in the Coronal portion of Ultrasonic group.

Figure-15 shows the image of Group I (Ultrasonic) which was obtained in middle portion for the percentage of penetration of root canal sealers into dentinal tubules.

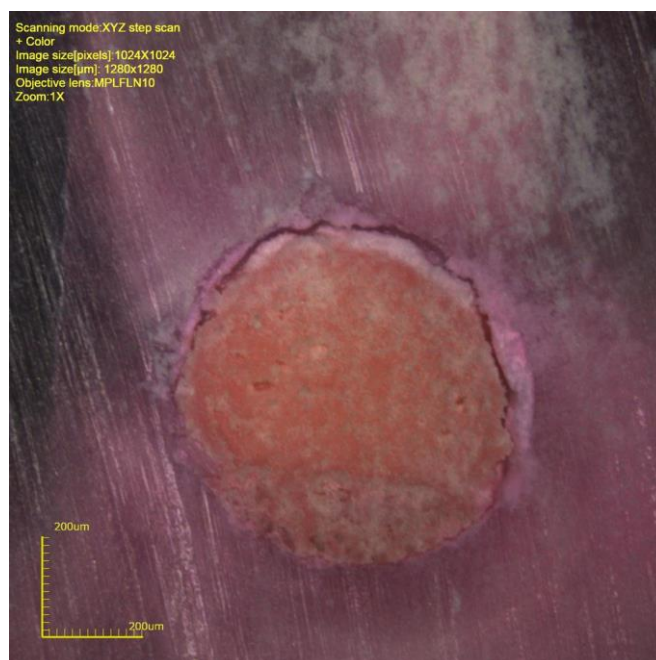


Figure 15- Percentage of penetration of root canal sealers into dentinal tubules in the middle portion of Ultrasonic group.

Figure-16 shows the image of Group I (Ultrasonic) which was obtained in the apical portion for the percentage of penetration of root canal sealers into dentinal tubules.

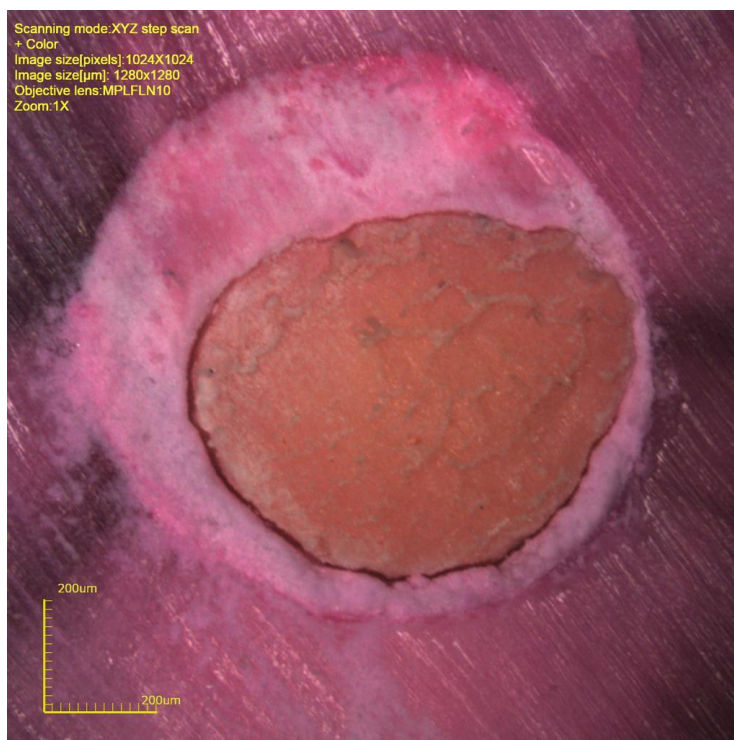


Figure 16- Percentage of penetration of root canal sealers into dentinal tubules in the apical portion of Ultrasonic group.

Figure-17 shows the image of Group II (Lentulo) which was obtained in the coronal portion for the percentage of penetration of root canal sealers into dentinal tubules.

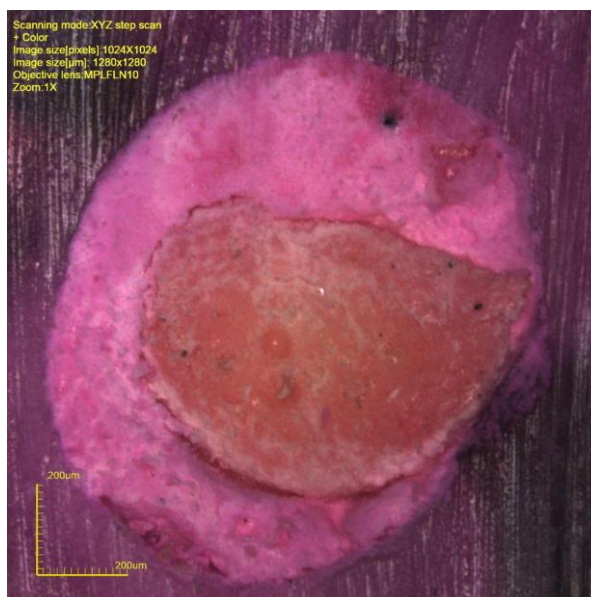


Figure 17- Percentage of penetration of root canal sealers into dentinal tubules in the coronal portion of Lentulo group.

Figure-18 shows the image of Group II (Lentulo) which was obtained in the middle portion for the percentage of penetration of root canal sealers into dentinal tubules.

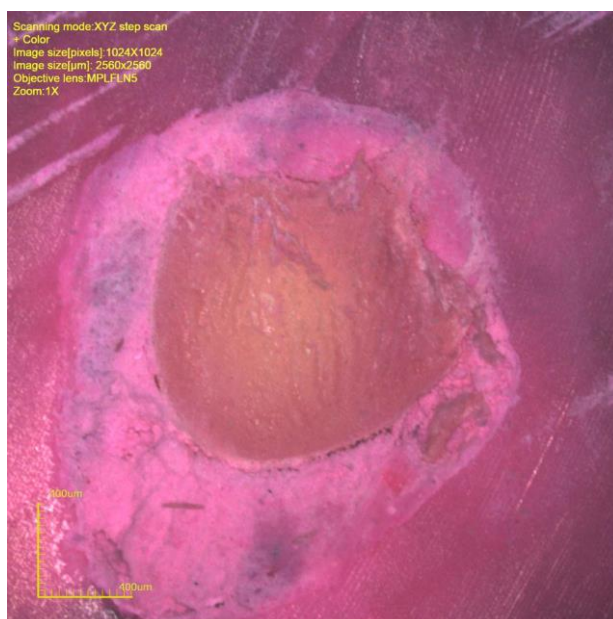


Figure 18- Percentage of penetration of root canal sealers into dentinal tubules in the middle portion of Lentulo group.

Figure-19 shows the image of Group II (Lentulo) which was obtained in the apical portion for the percentage of penetration of root canal sealers into dentinal tubules.

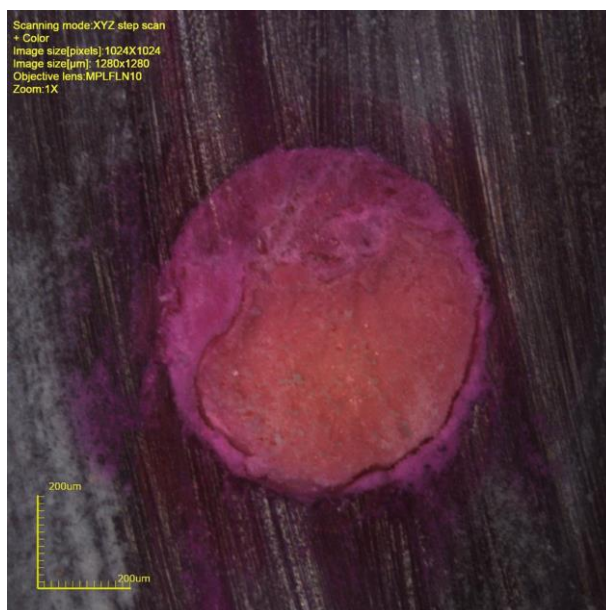


Figure 19- Percentage of penetration of root canal sealers into dentinal tubules in the apical portion of Lentulo group.

Figure-20 shows the image of Group III (Gutta percha) which was obtained in the coronal portion for the percentage of penetration of root canal sealers into dentinal tubules.

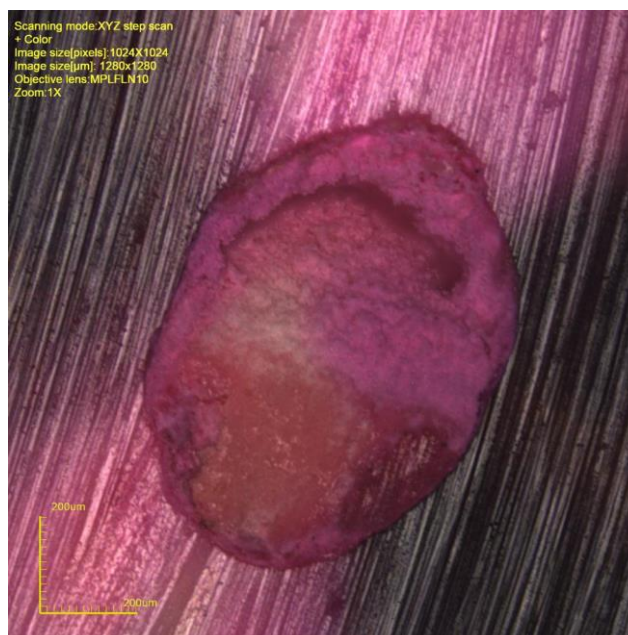


Figure 20- Percentage of penetration of root canal sealers into dentinal tubules in the coronal portion of Guttapercha group.

Figure-21 shows the image of Group III (Gutta percha) which was obtained in the middle portion for the percentage of penetration of root canal sealers into dentinal tubules.

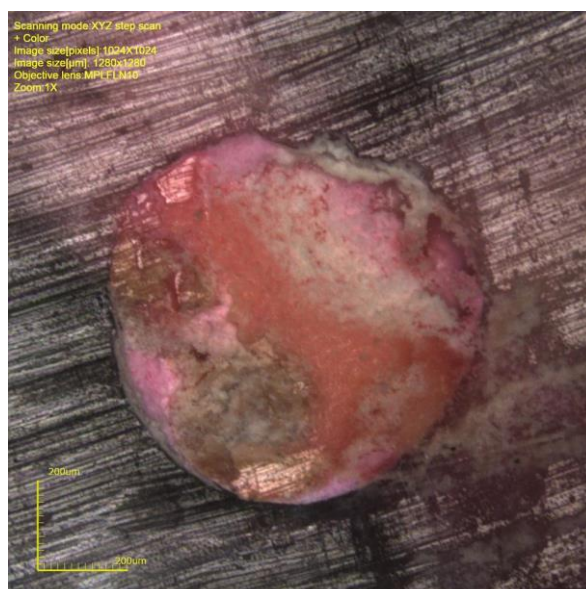


Figure 21- Percentage of penetration of root canal sealers into dentinal tubules in the middle portion of Guttapercha group.

Figure-22 shows the image of Group III (Gutta percha) which was obtained in the apical portion for the percentage of penetration of root canal sealers into dentinal tubules.



Figure 22 - Percentage of penetration of root canal sealers into dentinal tubules in the apical portion of Guttapercha group.

STATISTICAL ANALYSIS:**Table 3** – Mean and standard deviation for the Depth of penetration of root canal sealers into dentinal tubules**Descriptive Statistics**

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Ultrasonic	90	373.64	650.24	505.0701	6.17243	58.55683
Lentulo	90	322.54	661.48	466.3481	7.06129	66.98928
Guttapercha	90	.00	389.54	151.3637	11.60837	110.12664
Valid N (listwise)	90					

Table 4 – Mean and standard deviation for the percentage of penetration of root canal sealers into dentinal tubules**Descriptive Statistics**

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Ultrasonic_P	90	67.00	99.00	84.6667	.89999	8.53808
Lentulo_P	90	37.00	98.00	74.7667	1.42337	13.50327
Guttapercha_P	90	.00	82.00	39.5000	2.40226	22.78983
Valid N (listwise)	90					

Table 5- ANOVA for the Depth of penetration of root canal sealers into the dentinal tubules**ANOVA**

		Sum of Squares	df	Mean Square	F	Sig.
Ultrasonic	Between Groups	117875.233	2	58937.617	27.377	.000
	Within Groups	187297.035	87	2152.839		
	Total	305172.268	89			
Lentulo	Between Groups	51697.601	2	25848.801	6.468	.002
	Within Groups	347695.601	87	3996.501		
	Total	399393.203	89			
Guttapercha	Between Groups	69310.000	2	34655.000	2.985	.056
	Within Groups	1010071.051	87	11610.012		
	Total	1079381.051	89			

Table 6: Tukey HSD for the Depth of penetration of root canal sealers into the dentinal tubules**Multiple Comparisons**

Tukey HSD

Dependent Variable	(I) GROU P	(J) GROU P	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound

Ultrasonic	1	2	50.22500*	11.98009	.000	21.6587	78.7913
		3	88.37267*	11.98009	.000	59.8064	116.9390
	2	1	-50.22500*	11.98009	.000	-78.7913	-21.6587
		3	38.14767*	11.98009	.006	9.5814	66.7140
	3	1	-88.37267*	11.98009	.000	-116.9390	-59.8064
		2	-38.14767*	11.98009	.006	-66.7140	-9.5814
Lentulo	1	2	30.31533	16.32279	.157	-8.6060	69.2367
		3	58.69633*	16.32279	.002	19.7750	97.6177
	2	1	-30.31533	16.32279	.157	-69.2367	8.6060
		3	28.38100	16.32279	.197	-10.5404	67.3024
	3	1	-58.69633*	16.32279	.002	-97.6177	-19.7750
		2	-28.38100	16.32279	.197	-67.3024	10.5404
Guttapercha	1	2	33.69833	27.82087	.450	-32.6400	100.0366
		3	67.97467*	27.82087	.043	1.6364	134.3130
	2	1	-33.69833	27.82087	.450	-100.0366	32.6400
		3	34.27633	27.82087	.438	-32.0620	100.6146
	3	1	-67.97467*	27.82087	.043	-134.3130	-1.6364
		2	-34.27633	27.82087	.438	-100.6146	32.0620

*. The mean difference is significant at the 0.05 level.

Table 7 - ANOVA for the Percentage of penetration of root canal sealers into the dentinal tubules**ANOVA**

		Sum of Squares	df	Mean Square	F	Sig.
Ultrasonic_P	Between Groups	2308.267	2	1154.133	24.023	.000
	Within Groups	4179.733	87	48.043		
	Total	6488.000	89			
Lentulo_P	Between Groups	5550.067	2	2775.033	22.610	.000
	Within Groups	10678.033	87	122.736		
	Total	16228.100	89			
Guttapercha_P	Between Groups	12708.200	2	6354.100	16.494	.000
	Within Groups	33516.300	87	385.245		
	Total	46224.500	89			

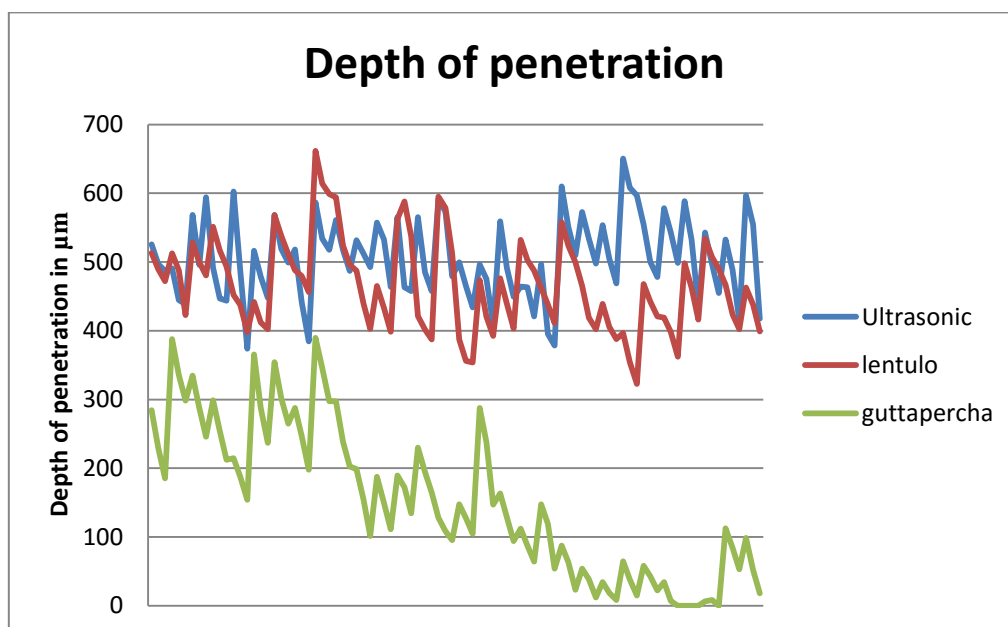
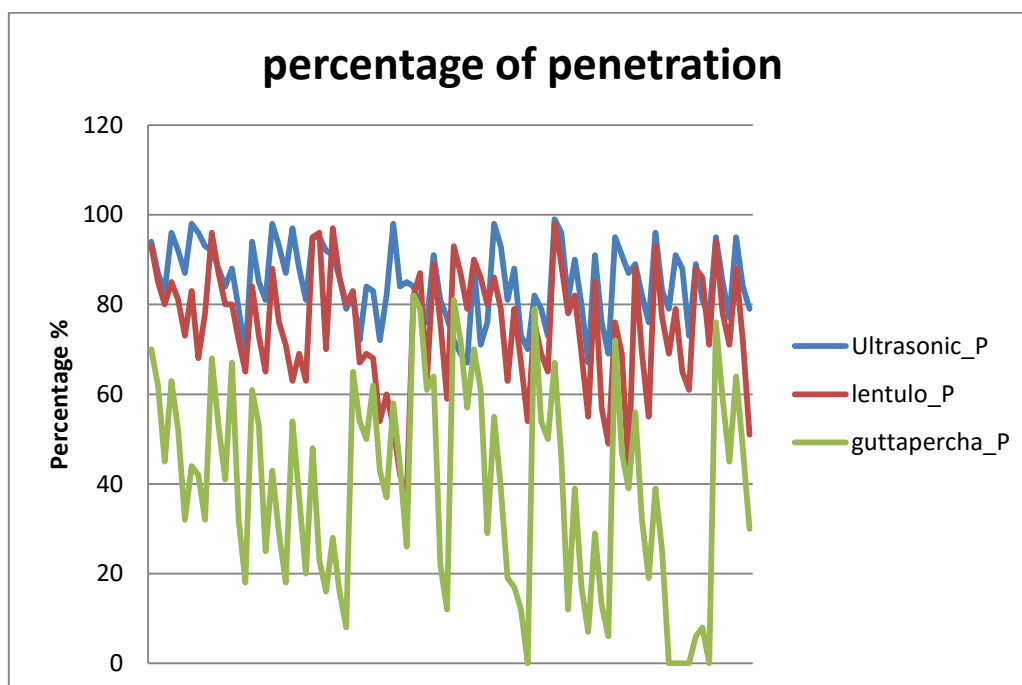
Table 8: Tukey HSD for the Percentage of penetration of root canal sealers**Multiple Comparisons**

Tukey HSD

Dependent Variable	(I) GROU P_P	(J) GROU P_P	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Ultrasonic_P	4	5	7.73333 [*]	1.78965	.000	3.4659	12.0007
		6	12.26667 [*]	1.78965	.000	7.9993	16.5341
	5	4	-7.73333 [*]	1.78965	.000	-12.0007	-3.4659
		6	4.53333 [*]	1.78965	.035	.2659	8.8007
	6	4	-12.26667 [*]	1.78965	.000	-16.5341	-7.9993
		5	-4.53333 [*]	1.78965	.035	-8.8007	-.2659
Lentulo_P	4	5	9.86667 [*]	2.86049	.002	3.0459	16.6874
		6	19.23333 [*]	2.86049	.000	12.4126	26.0541
	5	4	-9.86667 [*]	2.86049	.002	-16.6874	-3.0459
		6	9.36667 [*]	2.86049	.004	2.5459	16.1874
	6	4	-19.23333 [*]	2.86049	.000	-26.0541	-12.4126
		5	-9.36667 [*]	2.86049	.004	-16.1874	-2.5459
Guttapercha_P	4	5	15.10000 [*]	5.06784	.010	3.0158	27.1842
		6	29.10000 [*]	5.06784	.000	17.0158	41.1842
	5	4	-15.10000 [*]	5.06784	.010	-27.1842	-3.0158
		6	14.00000 [*]	5.06784	.019	1.9158	26.0842

6	4	-29.10000*	5.06784	.000	-41.1842	-17.0158
	5	-14.00000*	5.06784	.019	-26.0842	-1.9158

*. The mean difference is significant at the 0.05 level.

Line Diagram 1: Depth of penetration of root canal sealers in μm **Line Diagram 2: Percentage of penetration of root canal sealers in %**

The mean and standard deviation of the depth of penetration are presented in Table 3.

ANOVA and **Tukey HSD** values for depth of penetration were presented in Table 5 and Table 6.

Figure 1 to 9 Shows representative of Confocal laser scanning microscopic images at 20X for the Depth of penetration of root canal sealers.

Depth of Penetration:

The results of the present study showed that the Ultrasonic group(Group I) had statistically significant difference in the depth of penetration at apical(3mm), middle(6mm) and coronal 9mm levels when compared to the lentulospiral group (Group II) and Guttapercha group (Group III) (P value is < 0.05 ; ANOVA-Tukey HSD).

The Lentulo group (Group II) showed a statistically significant difference in the depth of penetration at apical (3mm), middle (6mm) and coronal (9mm) levels when compared to the Guttapercha group (Group III)

ULTRASONIC GROUP (GROUP I):

When comparing within groups, the coronal third (9mm) showed a statistically significant difference in the depth of penetration of root canal sealers when compared to the middle (6mm) and the apical third (3mm) of the root canal. (P value is < 0.05 ; ANOVA-Tukey HSD).The middle third (6mm) did not show a statistically significant difference when compared to the apical third (3mm).(P value is > 0.05 ; ANOVA-Tukey HSD)

LENTULO GROUP (GROUP II):

When comparing within groups the coronal third (9mm) showed a

Statistically significant difference in the depth of penetration of root canal sealers when compared to the apical third (3mm) of the root canal.(P value is < 0.05 ; ANOVA-Tukey HSD).There was no significant difference in the middle third (6mm) of the root canal. (P value is >0.05 ; ANOVA-Tukey HSD).

GUTTAPERCHA GROUP (GROUP III):

When comparing within groups, the coronal third (9mm) does not show a statistically significant difference in the depth of penetration of root canal sealers when compared to the middle third (6mm) and the apical third (3mm) of root canal.(P value is < 0.05 ; ANOVA-Tukey HSD).

PERCENTAGE OF PENETRATION OF THE ROOT CANAL SEALER:

The mean and standard deviation of the percentage of penetration of sealer are presented in Table 4.

ANOVA and **Tukey HSD** values for the percentage of penetration of the root canal sealer have been presented in Table 7 and Table 8.

Figures 10 to 18 show the Confocal laser scanning microscopic images (10X) of the percentage of penetration of the root canal sealer.

Ultrasonic group(Group I) showed statistically significant difference in percentage of penetration at the apical(3mm) , middle(6mm) and the coronal (9mm) third of the root canal when compared to the Lentulospiral group (Group II) and the Guttapercha group (Group III) (P value is < 0.05 ; ANOVA-Tukey HSD).

The lentulo group (Group II) showed statistically significant difference in the percentage of penetration of root canal sealer at the apical (3mm), middle (6mm) and the coronal (9mm)

third when compared to the Guttapercha group (Group III) (P value is < 0.05 ; ANOVA-Tukey HSD).

ULTRASONIC GROUP (GROUP I):

When comparing within groups, the coronal third (9mm) showed statistically significant difference in the percentage of penetration of root canal sealer when compared to the middle (6mm) and the apical third(3mm) of root canal.(P value is < 0.05 ; ANOVA-Tukey HSD).

LENTULO GROUP (GROUP II):

When comparing within groups, the coronal third (9mm) showed statistically significant difference in the percentage of penetration of the root canal sealer when compared to the middle (6mm) and the apical third(3mm) of root canal.(P value is < 0.05 ; ANOVA-Tukey HSD).

GUTTAPERCHA GROUP (GROUP III):

When comparing within groups, the coronal third (9mm) showed statistically significant difference in the percentage of penetration of root canal sealers when compared to the middle third (6mm) and the apical third (3mm) of the root canal.(P value is < 0.05 ; ANOVA-Tukey HSD).

The sealer displayed different amounts of penetration into the dentinal tubules in the present study. The ultrasonic group (Group I) showed the highest depth of penetration with uniform sealer penetration in all three regions (i.e) coronal (9 mm), middle (6 mm), apical (3 mm) third of root canal. The lentulo group showed uniform sealer penetration in the coronal (9 mm) and middle (6 mm) third but showed poor penetration in the apical third (3

mm) of root canal. The Guttapercha group showed poor penetration of root canal of sealer in coronal (9mm), middle (6mm) and apical third (3mm).

DISCUSSION

Discussion:

The major goal of root canal filling is to prevent any interchange between the oral cavity, the root canal system, and the periradicular tissues, thus providing a barrier to canal infection and re-infection.

Sealers are used to attain an impervious seal between the core material and root canal walls. Sealer penetration into the dentinal tubules increases the interface between the obturating material and the dentin. The increase in the interface improves the sealing ability of the obturation. The removal of the smear layer from the root canal walls is regarded as an essential step of root canal treatment.^[36,37] Sealers are necessary to seal the space between the dentinal wall and the obturating core interface. In addition, they often have the ability to penetrate areas such as lateral canals and dentinal tubules. This property is highly relevant because the penetration of sealer cements into dentinal tubules increases their surface contact with the root canal dentin thus improving the sealing ability. Sealer plugs inside the dentinal tubules provide a mechanical interlocking, improving the retention of the filling material. The penetration of sealers into the dentinal tubules may be biologically beneficial, because laboratory studies have shown that endodontic sealers can exert antibacterial effects against bacteria in infected dentinal tubules.^[38] Bacterial penetration into the dentinal tubules may reach 100-1,000 μm and it can be enhanced by the absence of smear layer.^[39] Many species seen in the infection of the root canal have the propensity to penetrate deeply into the dentinal tubules, such as facultative and anaerobic species^[40-42]. These microorganisms penetrate upto the dentinal- cementum junction.^[43] Sealer cements may entomb any residual bacteria in the tubules rendering them harmless. The sealer would serve as a reasonable blocking agent that may prevent bacterial repopulation or inactivate them in the tubules. Further, it has been proposed that penetration of the sealer into the dentinal tubules may have a root strengthening effect due to filling of the voids.

Thus the ability of a sealer to penetrate into the dentinal tubules effectively may be one of the factors influencing the choice for selection of a sealer and sealer placement techniques during obturation. The apical 3 or 6 mm of a root canal is a critical area for the placement of a sealer. It is important for successful obturation because it is in this area that accessory canals are most often found. Since accessory canals communicate with the periodontal membrane, they can create a periodontic-endodontic pathway for potential bacterial penetration to and from the periodontium. ^[44, 45] Sealer placement technique performed using a combination of gutta-percha and a root canal sealer might not provide an optimal seal. ^[4]

This study was performed with Bioceramic sealer using three commonly used sealer placement techniques namely, ultrasonic, rotary and conventional sealer placement technique, the penetrability of Bioceramic sealer into the dentinal tubules and its percentage of penetration was evaluated at 3mm, 6mm and 9 mm (i.e., Apical, middle, cervical third respectively).

Bioceramic sealer was used in the present study because of its excellent physicochemical and biological properties. ^[57] It has low solubility and long term dimensional stability.

Studies performed using Lateral Condensation technique, showed that different sealer placement techniques did not interfere with the quality of the filling. However, one of the main disadvantages of lateral compaction technique has been the lack of a 3-dimensional root canal filling, especially in oval canals or irregular canals. ^[58] Lateral compaction shows contradictory results regarding the ability of sealers to penetrate into dentinal tubules. ^[59-61] In Single Cone method, Sealer placement technique plays a major role to produce a three

dimensional root canal filling without any voids. Hence in the present study we have used Single cone obturation method with three commonly used sealer placement techniques.

Several microscopy techniques are currently used to evaluate the sealer/dentin interface, including stereomicroscopy, SEM, TEM and, CLSM. Stereomicroscopy has a drawback of not being sensitive to extremely thin layers of sealer along canal walls, thus more sensitive detection techniques are used. CLSM offers several advantages over conventional SEM. It provides detailed information about the presence and distribution of sealers or dental adhesives inside the dentinal tubules in the total circumference of the root canal walls at relative low magnification (5X and 10X), through the use of fluorescent Rhodamine-marked sealers, artifacts could practically be excluded.^[46, 47] In addition it uses non-decalcified or hard tissue samples that do not require a specific section technique (sputter coating).

Thus the present study was carried out to assess the sealer-dentin interface and compare the percentage and depth of penetration of sealer into the dentinal tubules by using 10× & 20 × magnification in CLSM. In all the canals, the technique was directed with an attempt to duplicate the *in vivo* use of the sealer.

The results of this study indicate that all three methods of sealer placement may not consistently and completely cover dentinal walls after single cone obturation method. Although sealer was present in the majority of the areas examined, the 3-mm level demonstrated significantly less ($P < 0.05$) sealer coverage than 6-mm and 9-mm level. The coverage as well as the penetration of the sealer into the dentinal tubules was significantly greater ($P < 0.05$) at the 9-mm level than 6-mm and 3-mm level of the root canal irrespective of method of the sealer placement technique used. These results coincide with the studies performed by Weimann and Wilcox in 1996.^[48, 49] This could be due to the fact that the

number as well as diameter of tubules decreases on descending apically in the root canal. Furthermore, the apical portion of roots show pronounced variations in structures, for e.g., primary dentinal tubules are irregular in direction and density; some areas are devoid of tubules.^[50] Cementum-like tissue can line the apical root canal wall, occluding any tubules.^[51] The efficacy of the irrigant to remove the smear layer decreases towards the apical direction limiting the flow of sealer into the dentinal tubules.

Ultrasonic group (Group I) showed uniform distribution of the sealer irrespective of the region in the root canal than the Lentulo group(Group II) and Guttapercha group(Group III). Ultrasonic energy has the ability to create several nodes along the length of file.^[52] Poor percentage of sealer penetration and depth of sealer penetration is more in the apical region. This might be due to the activated file touching the canal wall in the more constricted area and not being able to produce the necessary nodes for acoustic streaming and cavitation.^[53] Ultrasonic instruments with their constant power supply and increased node production cannot effectively clean the more apical region. Significantly better percentage of sealer penetration and depth of sealer penetration was observed in ultrasonic group, substantiating the findings of previous studies.^[54, 55, 56] All these studies concluded that the use of ultrasonics results in better sealer placement than other compared techniques. The ultrasonic and sonic energy apparently propels the relatively viscous sealer along the length of file to an appropriate depth^[54] while lentulo spiral centrifugally pushes the sealer.

Another reason might be due to the ability of Bio-ceramic sealers to penetrate dentinal tubules even in the presence or absence of smear layer.

Previous studies have shown that the rotary lentulo spiral group will produce a better adaptation of the sealer onto the canal walls with even thickness which in turn leads to a better seal but the results of this study did not correlate with their findings. This may be

attributed to several factors. First, more amount of sealer was introduced into the canal as compared with other techniques, and as the sealer shrank during setting, more gaps and voids were created that contributed to the highest value of microleakage. Second, a high volume of the sealer material may also interfere with the placement of additional accessory points which lead to less gutta-percha volume percentage compared to the amount of sealer. Third, the use of rotary lentulo spiral during sealer placement may force some air bubbles into the material that will lead to void formation and microleakage, whereas, the endodontic sealer coating of master gutta-percha cone produced less sealer thickness with less potential for void formation compared to the other techniques that eventually might have contributed to the smallest microleakage value obtained by this group.

In this study the Gutta percha group (Group III) produced less sealer thickness around the canal walls, which has a high potential for void formation when compared to the other techniques which propels the root canal sealer into the dentinal tubules. Less sealer thickness produced around the canal walls in the Gutta percha group (due to the passivity of the technique) eventually might have contributed to the lowest penetration of root canal sealers into dentinal tubules.

Gutta percha group (Group III) showed that, irrespective of the region (3mm, 6mm and 9mm) , there was uneven circumferential distribution of root canal sealers around the root canal walls as well as poor depth of penetration into the dentinal tubules.

Within the limitations of this study it can be concluded that the depth and percentage of penetration of the sealer is influenced by the type of sealer placement technique and by the root canal level (coronal, middle and apical third) with penetration decreasing apically. All the analysed placement techniques failed to show a consistent adaptation of sealer to the total circumference of the root canal wall.

The ultrasonic group showed the highest mean in Depth of penetration into dentinal tubules and showed uniform distribution of sealer in coronal, middle and apical third of root canal, whereas the lentulo group showed uniform distribution in the coronal and middle third of root canal but in the apical third it showed poor depth of penetration and distribution of the sealer. Gutta percha group showed poor depth of penetration and distribution of sealer in the coronal, middle and apical third of root canal.

SUMMARY & CONCLUSION

SUMMARY:

The present study was conducted in the department of Conservative dentistry and Endodontics, KSR Institute of Dental Science and Research. After approval from institutional review board, a randomized controlled clinical trial was planned. Sixty maxillary Anterior teeth were obturated using Endosequence BC sealer with rhodamine B dye, using three different common sealer placement techniques (n=20) (i.e) Ultrasonic, Lentulospiral, Guttapercha and evaluated using Confocal laser scanning microscope at 10X for the percentage of penetration of root canal sealers and at 20X for the depth of penetration of root canal sealers into dentinal tubules.

The findings of the present study can be summarized as follows

1. There was a statistically significant difference in the depth and percentage of penetration of root canal sealers when using three different sealer placement techniques.
2. The ultrasonic group showed the highest mean in the depth of penetration and also showed a uniform circumferential distribution of sealer in all three regions i.e; the coronal (9mm), middle (6mm), and the apical third (3mm) of the root canal.
3. The lentulospiral group showed uniform circumferential distribution of sealer in the coronal (9mm) and the middle (6mm) third, but showed poor depth of penetration and poor distribution of sealer in the apical third (3mm) of root canal.
4. Guttapercha group showed poor circumferential distribution along the root canal walls and poor depth of penetration of sealer in all three regions i.e; the coronal (9mm), middle (6mm) and the apical third (3mm) of the root canal.

CONCLUSION:

The following inference has been derived from this study. The ultrasonic sealer placement technique is a reliable technique as it provides a three dimensional endodontic filling and it efficiently enhances the sealing ability of the root canal.

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.ANNEXURE



INSTITUTIONAL ETHICAL COMMITTEE

KSR INSTITUTE OF DENTAL SCIENCE & RESEARCH

KSR Kalvi Nagar, Tiruchengode-637 215, Tamilnadu.
Phone : 04288-274981, Fax : 04288-274761,
email : ksr dentalcollege@yahoo.com

Chairman

Dr. P. PONMURUGAN, Ph.D.,

Prof. & Head Dept. of Biotechnology
KSR College of Technology,
KSR Kalvi Nagar, Tiruchengode.

Member Secretary

Dr. G.S. KUMAR, MDS.,

Principal,
KSR Institute of Dental Science & Research,
KSR Kalvi Nagar, Tiruchengode.

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Mr.A.Thirumoorthi, M.A.B.L.,
Human Activist

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Family Counsellor

Dr.K.Sivakumar, MDS., (Cons Dent.)

Dr.Suman, M.D.S., (OMDR)

Dr.Sharath Ashokan, MDS., (Pedo)

Dr.G.Rajeswari, Ph.D., (Biochemistry)

Dr.K.Karthick, MDS., (Cons Dent.)

Mr.V.Mohan, M.Sc., M.Phil., (Physicist)

Mr.A.P.S.Raja, B.A.,
(Layperson)

Ref.: 068 /KSRIDSR/EC/2014

Date : 09.01.2014

To

Dr. S.Kumar,
Postgraduate Student,
Dept. of Conservative Dentistry & Endodontics,
KSR Institute of Dental Science & Research,

Your dissertational study titled "DEPTH & PERCENTAGE OF PENETRATION OF BIO-CERAMIC SEALERS INTO DENTINAL TUBULES AFTER ULTRASONIC, ROTARY, CONVENTIONAL SEALER PLACEMENT TECHNIQUE - A CONFOCAL LASER SCANNING MICROSCOPIC STUDY" presented before the ethical committee on 7th Jan. 2014 has been discussed by the committee members and has been approved.

You are requested to adhere to the ICMR guidelines on Biomedical Research and follow good clinical practice. You are requested to inform the progress of work from time to time and submit a final report on the completion of study.


Signature of Member Secretary,
(Dr.G.S.Kumar)